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Response surface optimization of formulation condition for development low-fat pork patties using different fat mimetics

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Abstract
The objectives of the present study were to optimize the level of incorporation of microcrystalline cellulose (MCC), non-fat dry milk powder (NFDM) and olive oil for the development of low-fat pork patties. Three coded levels for MCC (1%, 2% and 3%), NFD (1.5%, 3% and 4.5%) and olive oil (3%, 7% and 11%) were analysed using Response Surface Methodology and 17 different runs were generated for the development of low-fat pork patties. These combinations were evaluated for cooking yield, emulsion stability, hardness, L*, *a**, *b**, juiciness and overall acceptability. Design-Expert 12 software was used for the numerical optimization. All the experimental values of product parameters were comparable with the predicted values derived from the respective model of regression coefficient and suggested that these models are satisfactory and accurate. Standardized protocol of RSM exhibited that Low-fat pork patties can be developed by the inclusion of MCC, NFDM and OO at 2%, 3% and 7 % respectively with greater Cooking Yield, Emulsion Stability and Overall acceptability.

Keywords: Microcrystalline cellulose, olive oil, non-fat dry milk powder, response surface methodology

Introduction
Pork has long been regarded as a valuable source of B vitamins, particularly thiamin, along with proteins and certain inorganic elements (Moss *et al*., 1983) [24]. Because of the high biological value of proteins and micronutrients, meat products are key to a healthy balanced diet (Zhuang., 2016) [47]. Saturated fatty acids and cholesterol are plentiful in pork backfat, which is added to the comminuted meat product (Del Nobile *et al*., 2009) [8]. Additionally, during the manufacturing of comminuted meat products such as frankfurters, meat patties backfat up to the level of 20-30% can be incorporated (Han & Bertram, 2017) [12]. According to the World Health Organization, fat should constitute 15 to 30% of total calories ingested, saturated fat should not surpass 10% of total dietary energy, and cholesterol intake should not reach beyond 300 mg per day (Jiménez-Colmenero *et al*., 2001) [15]. Also, consumers become more health-conscious, there has been a transition in conventional eating patterns toward foods with lower fat, cholesterol, and calorie content, including meat products (Pietrasik & Janz, 2010) [36]. Fat contributes to the flavor which is a combination of taste and odors and develops primarily during the heating process (Brewer, 2012) [6]. In meat batter, fat-protein-water interaction play important role in stabilizing meat emulsion. The fat droplets are embedded and fixed in the protein matrix and also interact with other ingredients to improve the stability of the meat emulsion system as well as provide appropriate texture, flavor and juiciness (Zhao *et al*., 2019) [46]. All of these factors make fat an indispensable part of emulsified meat products and meat technologists confront a challenge in finding a suitable alternative for fat in meat products.
Low-fat meat products can be produced by eliminating intermuscular or external fat off the raw carcass and then using lean muscle cuts in the formulation; by genetic and dietary manipulation of animals to reshape fatty acid profiles and inclusion of fat replacers in processed meat products potentially reduce fat content (Keeton, 1994) [17]. A fat replacer is a substance that can perform the functionalities of fat while providing less calories and must be able to imitate all or some of the functional properties of fat in the modified foods (Omayma & Youssef, 2007) [30]. Fat replacers can be classified into fat mimetics and fat substitutes.

Fat mimetics are agents which imitate physical and organoleptic properties of fats but cannot completely replace fat. These substances are primarily carbohydrates or proteins that require hydration in undertaking their function as fat substitutes (Ospina-E *et al*., 2012) [31].

Microcrystalline cellulose is extensively utilized in the production of low-fat emulsions. It is a calorie-free polysaccharide hydrocolloid consisting of cellulose and carboxymethylcellulose particles that generates a fat-like gel in aqueous solutions (Zbikowska *et al*., 2018) [45]. Microcrystalline cellulose can serve a variety of functions in the fabrication of processed foods, including fat replacement, encapsulation, emulsion stabilization, edible film production and bulking agent (Nsor-Atindana *et al*., 2017) [28]. Microcrystalline cellulose is a source of dietary fibre that has numerous health benefits including the prevention of cardiovascular disease, the reduction of certain types of cancer, weight management, lower risk of type 2 diabetes mellitus, and the enhancement of gut health (Mehta *et al*., 2015) [21]. Microcrystalline cellulose is incorporated in various meat products such as emulsified sausage (Schuh *et al*., 2013) [41], beef emulsion (Mejia *et al*., 2019) [43], pork patties (Todd *et al*., 1990) [42] etc. The US Food and Drug Administration describes non-fat dry milk powder as obtained by drying water from pasteurised skim milk and containing 5% or less moisture and 1.5% or less milkfat (ADPI, 2015) [1]. Milk proteins have notable functional qualities such as solubility, water holding capacity, viscosity, color enhancement, gelation, and emulsion stablization (Kumar & Sharma, 2003) [18]. NFDM is incorporated in various meat products such as meat patties (Andic *et al*., 2010) [2], sausages (Ensor *et al*., 1987; Pagthinathan & Gunasekara, 2021), meat emulsion (Parks & Carpenter., 1987) [10] etc. Olive oil is monounsaturated vegetable oil, containing 56-87% monounsaturated fatty acids (MUFA), 8-10% saturated fatty acids (SFA) and 10-22% polyunsaturated fatty acids (PUFA) (IOOC, 1984) [14]. Olive oil is rich in antioxidants, has a greater biological value, lesser saturated fatty acids, and provides alpha linolenic acid which precursor to longer chain unsaturated omega-3 fatty acids like EPA and DHA (Muguerza *et al*., 2002) [25]. Olive oil is incorporated in various meat products such as frankfurters (Paneras & Bloukas, 1994) [33], sausages (Pintado & Cofrades, 2020) [37], pork patties (Rodríguez-Carpena *et al*., 2012) [39] etc.

Material and Methodology

Source of Pork, Non-Meat Ingredient and Additive

Castrated Large White Yorkshire pig of nine-month-old weighing 70 Kg was procured from University Pig Farm, Department of Livestock Production Management, GADVSU, Ludhiana. The slaughtering of the pig was done as per standard protocol in an experimental slaughterhouse of the Department of Livestock Product Technology (LPT), College of Veterinary Science, GADVASU, Ludhiana, Punjab; with due consideration of animal welfare aspects. The dressed carcass chilled at 4±1 °C for 12-18 hour in the product laboratory of the Department of LPT. Manual deboning of the carcass was done deboned pork packed in low-density polyethylene bags having a capacity of 1 kg and stored in a deep freezer at -18±1 °C for further use. The required quantity of frozen pork was taken out and thawed overnight in a refrigerator (4±1 °C) and proportioned into smaller chucks of nearly 1 square inch for further study.

Microcrystalline cellulose powder (MICCEL P) was procured from Ankit Pulps & Boards Pvt. Ltd. Nagpur, Maharashtra. Verka spray-dried skim milk powder packed in polyfilm pack of 500g and pomace olive oil (MUFA 75%, PUFA 10% and SFA 15%) of Oleev brand was used in the experiment. The other additives used were salt (Tata Chemicals Ltd., Mumbai India), STPP or tetrasodium pyrophosphate (Hi-media Laboratories Pvt. Ltd., Mumbai, India), and sodium nitrite (Central Drug House Pvt. Ltd., New Delhi, India).

Preparation of Pork Patties

Pork subjected to overnight partial thawing under refrigeration (4±1°C for 24 hrs) was cut into small cubes and double minced through a meat mincer (Mado Eskimo Mew-714, Mado Germany) using 4 mm plate. Pork emulsion was prepared in a bowl chopper (Seydelmann K20, Ras, Germany). Pre-weighed quantity of minced pork, salt (1.5%), sodium nitrite (100 ppm) and sodium tripolyphosphate (0.20%) and were added and chopping was done for about 2-3 minutes. Further, ice flakes were added and chopping was carried again for 2 minutes. Condiments paste (3%), dry spice mix (1.5%), refined wheat flour (3%) and egg (2.5%) and sugar (0.30%) were added. Chopping was continued till uniform dispersion of all the ingredients and desired consistency of the emulsion was accomplished. Pork emulsion was moulded into patties and cooked at 180^0C for 30 minutes. In each trial of the experiment different levels of microcrystalline cellulose, NFDM and olive oil added and pork backfat was replaced 100 percent in this experiment.

Analytical Methods

Instrumental Colour Profile Analysis

The Colour profile was measured using CR-400 Konica Chroma meter (Konica Minolta, Japan) set at 2° of cool white light (d_{65}) and known as '*L*', *a*, and *b* values. '*L*' value denotes (brightness 100) or lightness (0), *a* (+ redness/-greenness), *b* (+ yellowness/-blueness). The instrument was directly put on the surface of pork patties at three different points.

Instrumental Texture Profile Analysis

Instrumental texture profile analysis (TPA) was conducted using a Texture analyzer (TMS-PRO, Food Technology Corporation, USA). A sample size of 1.0cm, 1.0cm, 1.0cm was subjected to pre-test speed (30mm/sec), post-test speed (100mm/sec) and test speed (100mm/sec) to a double compression cycle with a load cell of 2500 N. A compression platform of 25 mm was used as a probe. The TPA was performed as per the procedure outlined by Bourne (1978). Parameter hardness was calculated automatically by the preloaded software in the equipment from the force-time plot.

Cooking Yield

The weight of each group product was recorded before and after cooking. The cooking yield was calculated and expressed as percentage by a formula

$$\text{Cooking yield (\%)} = \frac{\text{Wt. of cooked product}}{\text{Wt. of raw pork emulsion}} \text{ X100}$$

Emulsion Stability

For calculation of Emulsion stability, 20 g of pork meat emulsion was taken in low density polyethylene (LDPE) bags of 150 gauge (size 11x10 cm) and were placed in a thermostatically controlled water bath (Equitron, Model: 8414, Medica Instrument Mfg. Co., Mumbai, India) at 80±1 ºC for 20 minutes. Thereafter, the bags were removed from the water bath, drained off the fluid (fat, water soluble solids) and weight of the cooked mass was recorded. The cooked emulsion was weighed and expressed as percentage (Baliga and Madaiach, 1970) [3].

Sensory Analysis

The sensory panel consisted of seven experienced and trained members selected among the pool of faculty members and postgraduate students. The sensory panel evaluated the developed low-fat pork patties for attributes of juiciness and overall acceptability using on a 5-point hedonic scale ranging from 1 as extremely undesirable to 5 as extremely desirable. The samples were evaluated in three sittings for this experiment, and 21 observations were recorded.

Experimental design

Response surface methodology (RSM) used to optimize the level of incorporation of microcrystalline cellulose, non-fat dry milk powder and olive oil as three compositions coded/uncoded variables (Table 1). The experiments were formulated according to Box-Behnken Design (BBD) resulting in 17 experimental designs of independent variables (Table 2). To develop the most acceptable low-fat pork patties microcrystalline cellulose, non-fat dry milk powder and olive oil responses were demonstrated by various parameters viz. Cooking yield, Colour analysis (L, a* and b*), texture analysis (Hardness) and Sensory analysis (Juiciness, Overall Acceptability; OA). The fitness of the polynomial model to the responses was evaluated by the Coefficient of R square as well as by the lack of fit using the F- test with 5% level of significance.

Table 1: Coded and uncoded levels of independent variables used in the Box-Behnken Design (BBD) for low-fat pork patties

Symbols	Independent variables	Coded levels		
		-1	0	+1
A	Non-fat dry milk powder (NFDM)	1.5	3	4.5
B	Microcrystalline cellulose (MCC)	1	2	3
C	Olive oil (OO)	3	7	11

Table 2: Experimental design of independent variables to development of low-fat pork patties.

Run	Independent variables (Factors)		
	NFDM	MCC	Olive oil
1	3	1	3
2	3	1	11
3	3	3	11
4	1.5	2	3
5	3	2	7
6	1.5	1	7
7	1.5	3	7
8	3	2	7
9	4.5	1	7
10	3	2	7
11	4.5	3	7
12	1.5	2	11
13	4.5	2	11
14	3	2	7
15	4.5	2	3
16	3	2	7
17	3	3	3

Result and Discussion

Fitting the Models

Each response was evaluated as a function of linear, quadratic and interaction effect of the independent variables viz. MCC, NFDM and olive oil. The experimental data were fitted into the second-order polynomial equation and the regression coefficient were calculated, the significance of the coefficient of the models was determined by analysis of variance (ANOVA) as summarised in Table. The quality of generated models was evaluated by ANOVA, R^2 and the lack of fit of the model. The ANOVA result in Table 3-4 and Fig 1-8 suggest that the model had very high F- values and very low p-value (<0.001) for all 7 studied responses. The Coefficient of variation (CV) describes the extent to which the data were dispersed. The CV values (ranging from 0.02- 3.31) for the proposed models indicated the high precision and reliability of the experiments.

In addition, high R^2 values and non-significant 'Lack of Fit' ($p>0.05$) were observed in Table 3-4 indicating that the studied model was highly significant to obtained data and capable of describing the relationship between the formulation conditions and studied responses. The larger regression coefficient in a model with a significant p-value indicated a more significant effect on the respective response variables.

The coefficients of multiple determinations (R^2) 0.1379, 0.7963, 0.9396, 0.6730, 0.9672, 0.9796 and 0.9969 were obtained for the response of overall acceptability, a*, *b**, juiciness, hardness, cooking yield and L* value respectively. It indicated that the second-order polynomial model was adequately represented by the respective experimental data.

Cooking Yield (CY)

The response surface analysis as shown in Table 5 demonstrated that the relationship between microcrystalline cellulose (MCC), non-fat dry milk powder (NFDM) and olive oil with relation to cooking yield with a good regression coefficient (R^2 = 0.9965) and model equation exhibited the relationship as per equation as follows.

$$CY = 84.11 + 0.5213A + 0.3363B - 0.0325C - 0.1125AB + 0.1350AC - 0.3200BC - 0.4173A^2 - 0.2223B^2 - 0.4597C^2$$

The ANOVA of the quadratic regression model showed that the model was significant (p<0.05) with a p value of <0.001. The response analysis revealed that NFDM, MCC and Olive oil had significant linear, quadratic and interaction effects on the CY of developed low-fat pork patties. Low CV (0.04) indicate higher precision and reliability of the experiment. Non-significant relation to pure error value revealed by lower 'Lack of fit value (0.0528).

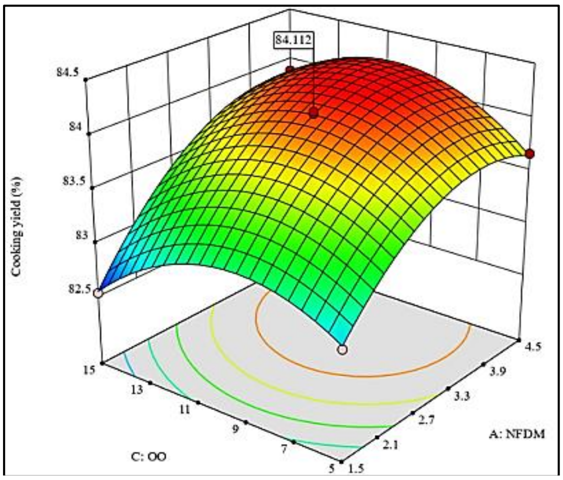

Fig 1: 3D Response surface plot for cooking yield

3D graph (Fig. 1) depicted the effect of Olive Oil, NFDM and MCC on the Cooking Yield of developed Low fat pork patties. The increase (p<0.05) in cooking yield was observed with linear increase in MCC. This could be because fibres (dietary) have a high-water binding ability (Gibis *et al*., 2015) [11]. Hydrocolloids such as MCC have hydrophilic groups with a water-binding site that attract the surrounding water subsequently reducing cooking loss and improving the cooking yield in the finished product. A similar finding was reported by Gibis *et al*. (2015) [11] reporting increase in MCC concentration subsequently resulted in a reduced cooking loss in beef patties. It is evident from Fig. 1 that linear increase in the level of incorporation of NFDM resulted in (p<0.05) an increase in Cooking Yield in the developed Low-fat pork patties. These findings are in agreement with those of Hung and Zayas (1992) [13], who found that NFDM had a very high emulsifying capacity and a good water holding capacity in comminuted meat products.

Olive oil significantly increased the cooking yield of developed low-fat pork patties. This might be due to the reason that olive oil when added to replace fat in meat batter entrap the part of water and fat in the matrix and resulted in less water loss during processing and improve the cooking yield of the product (Ruiz-Capillas *et al*., 2013) [40].

Table 5: ANOVA of the second-order polynomial model for the response variables viz. cooking yield, Hardness, Overall Acceptability, Juiciness

Source	DF	Cooking Yield (%)			Hardness			Overall Accept.			Juiciness		
		Coefficient	Sum of squares	p-Value	Coefficient	Sum of Squares	p-Value	Coefficient	Sum of squares	p-Value	Coefficient	Sum of squares	p-Value
Model	9	84.11	5.64	<0.0001	19.01	15.04	<0.0001	8.06	4.84	0.0053	7.53	0.8360	0.0001
Linear													
A (NFDM)	1	0.5213	2.17	<0.0001	0.9288	6.90	<0.0001	0.3062	0.7503	0.0112	-0.0112	0.0010	0.6043
B (MCC)	1	0.3363	0.9045	<0.0001	0.6288	3.16	<0.0001	-0.1212	0.1176	0.2184	0.0325	0.0085	0.1610
C (Olive oil)	1	-0.0325	0.0084	0.0339	0.0800	0.0512	0.0193	-0.3375	0.9112	0.0070	-0.2263	0.4095	<0.0001
Quadratic													
A B	1	-0.1125	0.0506	0.0004	-0.1425	0.0812	0.0067	0.0325	0.0042	0.8051	0.0400	0.0064	0.2148
A C	1	0.1350	0.0729	0.0001	0.3000	0.3600	<0.0001	0.5700	1.30	0.0028	-0.1375	0.0756	0.0022
B C	1	-0.3200	0.4096	<0.0001	0.4700	0.8836	<0.0001	-0.2300	0.2116	0.1126	-0.0250	0.0025	0.4221
Interaction													
A^2	1	-0.4173	0.7330	<0.0001	-0.4263	0.7650	<0.0001	-0.0618	0.0161	0.6327	-0.2587	0.2819	<0.0001
B^2	1	-0.2223	0.2080	<0.0001	-0.3863	0.6282	<0.0001	-0.2818	0.3342	0.0567	-0.0762	0.0245	0.0321
C^2	1	-0.4597	0.8900	<0.0001	-0.6637	1.86	<0.0001	-0.5092	1.09	0.0045	-0.0438	0.0081	0.1697
Residual	7		0.0086			0.0392			0.4504			0.0241	
Lack of fit	3		0.0071	0.0528		0.0300	0.0947		0.3679	0.0590		0.0169	0.1499
Pure error	4		0.0015			0.0092			0.0825			0.0072	
Total	16		5.65			15.08			5.29			0.8600	
Adj. R^2		0.9965			0.9941			0.8053			0.9360		
Pred. R^2		0.9796			0.9672			-0.1379			0.6730		
C.V. %		0.0418			0.4087			3.31			0.7977		

Hardness

RSM (Table 5) demonstrated the relationship between hardness with three independent variables viz. MCC, NFDM and olive oil in the developed low-fat pork patties. The following second-order polynomial equation was derived:

$$Hardness = 9.01+0.9288A+0.6288B+0.800C-0.1425AB+0.3000AC+0.4700BC-0.04263A^2-0.3863B^2-0.6637C^2$$

The R square value (0.9941) expressed that 99.41 % of the total variation reveals a more significant (p<0.05) effect of the variable on the hardness of the low-fat pork patties. It was observed in present experiment that NFDM, OO and MCC had a significant linear, quadratic and interactive impact on the firmness of developed LFPP. Low CV (0.4087) indicate higher precision and reliability of the experiment.

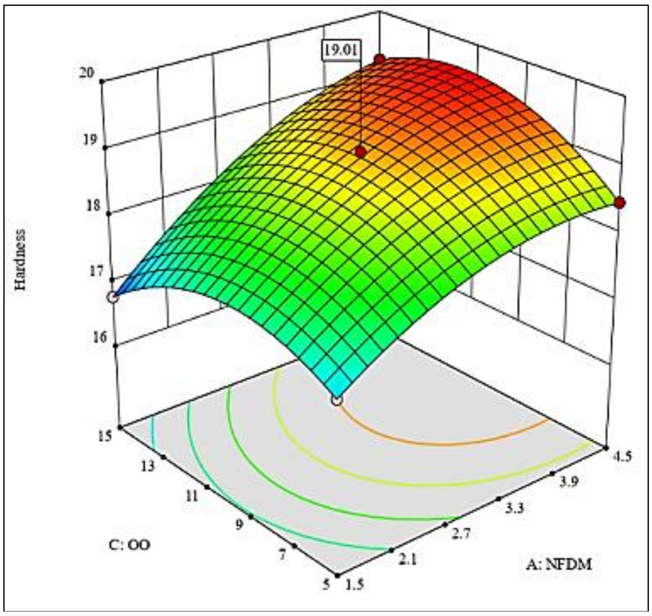

Fig 2: 3D Response surface plot for hardness

The Fig 2 showed the combined effect of NFDM, MCC and olive oil on the hardness of developed low-fat pork patties. It was observed that with an increasing MCC from 1% to 3% hardness of the patties increased significantly (p<0.05). The gelling properties of added MCC and entrapment of water and fat in the fibre matrix increased the hardness in the product (Han & Bertram, 2017) [12]. Mejia *et al* (2019) [43] reported that MCC produced stronger gel in the meat emulsion resulting in increased hardness. Similar findings were reported with NFDM addition in low-fat pork patties. Youssef and Barbut (2010) [44] reported incorporation of milk proteins in increased the hardness of the beef batter. The result of the 3D graph suggests that olive oil improved the hardness of the product. The combination of oil and non-meat protein results in better gel formation and oils have better distribution in meat batter as compared to animal fat (Delgado-Pando *et al* 2010) [9].

The optimum value for the hardness of low-fat pork patties was reported 19.01 as predicted by RSM by the use of three independent variables: NFDM (3%), MCC (2%) and olive oil (7%).

L* Value

L* value of developed low-fat pork patties is presented in table 6 and graphically in Fig. 3 The second-order polynomial equation generated between L* value and three independent variables (NFDM, MCC and Olive oil) is as under: -

$$L^* = 63.91+ 0.2625A + 0.8213B + 0.4312C - 0.0475AB - 0.2975AC - 0.0750BC + 0.0370A^2 - 0.1805B^2 - 0.1955C^2$$

From ANOVA of the regression coefficient, it was found that the model was significant with p value of <0.001 for L* value of developed pork patties. NFDM, MCC and olive oil have significant linear, quadratic and interaction effect on the L* value of developed low-fat pork patties. The R^2 value (0.9995) revealed that 99.95% of the total variation results more significant (p<0.05) effect of the variable on L* value indicating proper fit to the experimental data. A low value of CV (0.0255) indicated higher precision and reliability of the experiment. The lack of fit F- value 0.0530 signifies that lack of fit is not significant relative to the pure error.

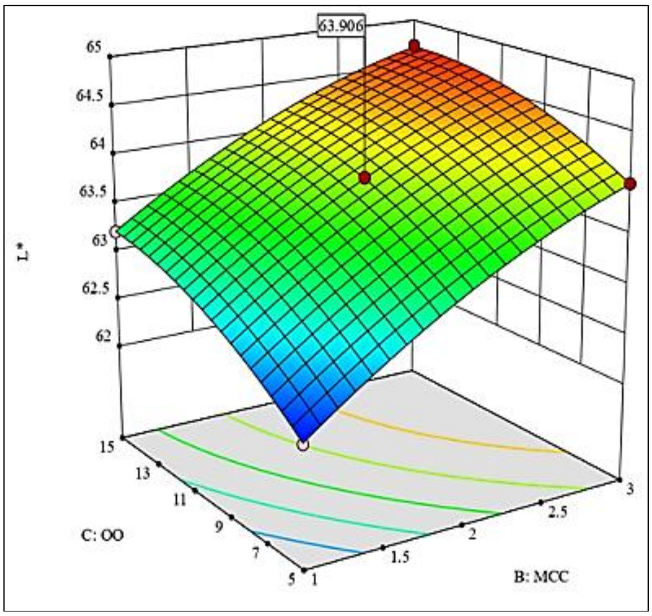

Fig 3: 3D Response surface plot for L*

It was observed that the L* value increased significantly (p<0.05) with an increasing trend of NFDM levels. This was might be due to bright white colour of added NFDM. The addition of olive oil results in an increase in the L* value of the low-fat pork patties. The result was par with the study conducted by Jiménez-Colmenero *et al* (2010) [16] where the replacement of olive oil in water emulsion increased lightness of developed sausages. It is evident from Fig.3 that linear increase in MCC addition increased the L* value in the developed pork patties. The results were in accordance with the study conducted by Schuh *et al* (2013) [41] where rising L* observed with the increase in concentration of MCC in emulsified sausage. Mittal & Barbut (1993) [22] reported that in low fat sausage with or without gums, L* values were increased because of less fat oozing from the leaner sausages during cooking resulting in less burning of fat on the product and lighter colour of product.

The optimum value for the L* of low-fat pork patties were reported 63.91 as predicted by RSM by the use of three independent variables: NFDM (3%), MCC (2%) and olive oil (7%).

4.4 *b** value

The *b** value of developed low fat pork patties is presented in table 6 and graphically in Fig.4 The second order polynomial equation generated between b* value and three independent variables (NFDM, MCC and Olive oil) is as under:

$$b^* = 12.83+0.1613A+ 0.3863B + 1.3900C - 0.1300AB + 0.1075AC - 0.1525BC- 0.2275A^2 -0.3325B^2-0.4500C^2$$

The ANOVA of the quadratic regression model showed that the model was significant ($p<0.05$) with p value of <0.001 for b* value. The R^2 value (0.9896) revealed that 98.96% of the total variation results more significant ($p<0.05$) effect of the variable on b* value indicating proper fit to the experimental data. A low value of CV (0.4409) indicated higher precision and reliability of the experiment. The statistical analysis revealed that NFDM, MCC and olive oil had significant linear and interactive effect on the b* value. The quadratic effect of NFDM and MCC; MCC and olive oil had a significant effect on the *b** value while the quadratic effect of NFDM and olive oil was not significant. The lack of fit F- value - 0.0649 signifies that lack of fit is not significant relative to the pure error.

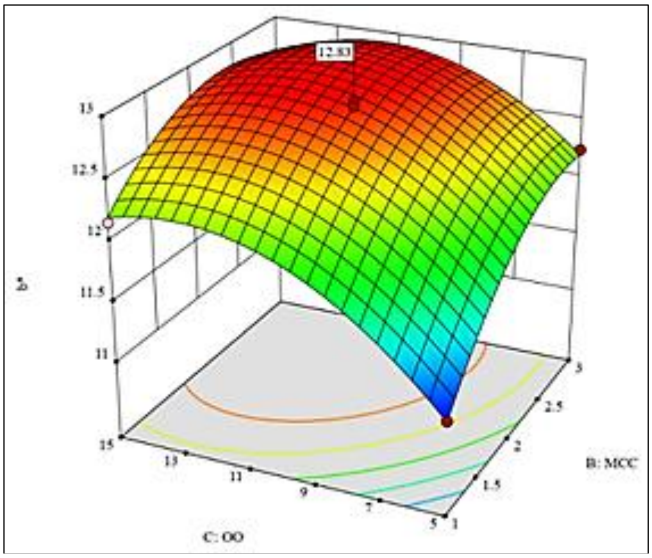

Fig 4: 3D Response surface plot for b*

Fig 4 pointed out that increasing the level of olive oil resulted in increased *b** value in developed low-fat pork patties. The difference may be attributed to the yellowish colour of the olive oil as compared to the white colour of pork fat (Jiménez-Colmenero *et al* 2010) [16]. The *b** value of developed low-fat pork patties significantly increased linearly with increasing levels of MCC in the low-fat pork patties. The increasing NFDM levels from 1.5 to 4.5 % had a significant increasing trend in *b** value of the low-fat pork patties. Barbut (2010) [44] reported that addition of skim milk powder and whey powder resulted in increased in *b** value in chicken meat.
However, optimum *b** value for the low-fat pork patties was recorded as 12.83 as predicted by RSM by the use of three independent variables: NFDM (3%), MCC (2%) and olive oil (7%).

4.5 *a** value

RSM (table 6) demonstrated the relationship between a* with three independent variables viz. MCC, NFDM and olive oil in the developed low-fat pork patties. The second order polynomial equation generated pertaining to a* value is depicted below;

$$a^* = 7.45 - 0.0675A -0.0337B - 0.0487C - 0.0100AB +0.0000AC +0.0025BC - 0.0278A^2 -0.0003B^2- 0.0352C^2$$

In present study it was observed that *a** value shows quadratic relationship with studied three variables i.e. NDFM, MCC and olive oil. The ANOVA of the quadratic regression model showed that the model was significant ($p<0.05$) with p value of 0.0003 for *a** value. The R^2 value (0.9151) revealed that 91.51% of the total variation results more significant (0<0.05) effect of variable on *a** value indicating proper fit to the experimental data. The NDFM, MCC and olive oil had significant linear effect on *a** value of the product. The statistical analysis showed that the quadratic effects of three variables on the a* value was not significant. The ANOVA table showed that there was non-significant interactive effect of MCC on *a** value. The lack of fit F- value of 0.4968 signifies that lack of fit is not significant relative to pure error.

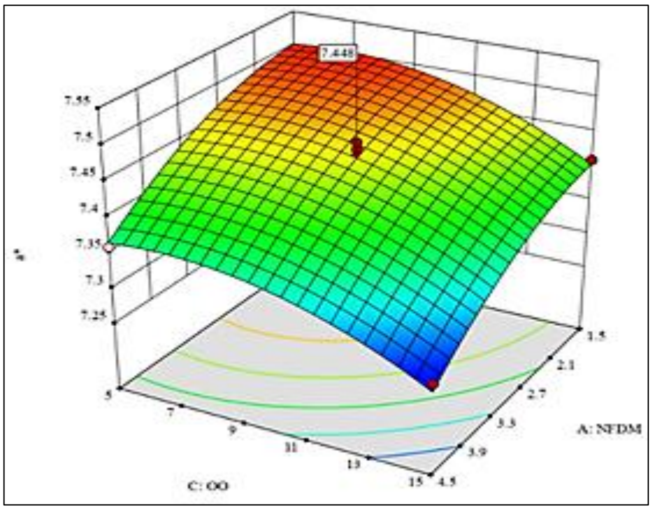

Fig 5: 3D Response surface plot for a*

3D graph shows the effect of NFDM, MCC and olive oil on *a** value of developed low-fat pork patties. The addition of olive oil resulted in decrease in *a** value in developed low fat pork patties. These findings were in consonance with Jiménez-Colmenero *et al* (2010) [16] where olive oil was incorporated in pork frankfurters resulting decrease in *a** value. It is evident from Fig. 5 that increasing levels of MCC had least effect on *a** value. The result was in accordance with study conducted by Schuh *et al* (2013) [41], where addition of MCC and Carboxymethyl cellulose showed least effect on the *a** value of the developed product. Similar findings were reported with addition of the NFDM. Youssef & Barbut (2010) [44] reported that addition of milk proteins to the meat batter resulted in decreased redness of the batter.
The optimum *a** value for the low-fat pork patties was recorded as 7.448 as predicted by studied RSM using three independent variables i.e. NFDM (3%), MCC (2%) and olive oil (7%).

Table 6: ANOVA of the second-order polynomial model for the response variables viz. L*, *a**, *b** and Emulsion stability.

Source	DF	L*			*a**			*b**			Emulsion Stability		
		Coefficient	Sum of squares	p-Value	Coefficient	Sum of Squares	p-Value	Coefficient	Sum of squares	p-Value	Coefficient	Sum of squares	p-Value
Model	9	63.91	8.14	<0.0001	7.45	0.0740	0.0003	12.83	4.52	<0.0001	87.49	5.68	<0.0001
Linear													
A (NFDM)	1	0.2625	0.5513	<0.0001	-0.0675	0.0364	<0.0001	0.1613	0.2080	<0.0001	0.5313	2.26	<0.0001
B (MCC)	*1*	*0.8213*	*5.40*	*<0.0001*	*-0.0337*	*0.0091*	*0.0021*	*0.3863*	*1.19*	*<0.0001*	*0.3263*	*0.8515*	*<0.0001*
C (Olive oil)	1	0.4312	1.49	<0.0001	-0.0487	0.0190	0.0002	0.3900	1.22	<0.0001	-0.0325	0.0085	0.0054
Quadratic													
A B	1	-0.0475	0.0090	0.006	-0.0100	0.0004	0.3550	-0.1300	0.0676	0.0020	-0.1075	0.0462	<0.0001
A C	1	-0.2975	0.3540	<0.0001	0.0000	0.0000	1.0000	0.1075	0.0462	0.0056	0.1350	0.0729	<0.0001
B C	1	-0.0750	0.0225	<0.0001	0.0025	0.0000	0.8116	-0.1525	0.0930	0.0008	-0.3200	0.4096	<0.0001
Interaction													
A^2	1	0.0370	0.0058	0.0023	-0.0278	0.0032	0.0258	-0.2275	0.2179	<0.0001	-0.4198	0.7419	<0.0001
B^2	1	-0.1805	0.1372	<0.0001	-0.0003	2.632E-07	0.9804	-0.3325	0.4655	<0.0001	-0.2248	0.2127	<0.0001
C^2	1	-0.1955	0.1609	<0.0001	-0.0352	0.0052	0.0090	-0.4500	0.8526	<0.0001	-0.4572	0.8803	<0.0001
Residual	7		0.0018			0.0029			0.0208			0.0038	
Lack of fit	3		0.0015	0.0530		0.0008	0.4968		0.0168	0.0649		0.0023	0.2496
Pure error	4		0.0003			0.0021			0.0040			0.0015	
Total	16		8.14			0.0768			4.55			5.68	
Adj. R^2		0.9995			0.9151			0.9896			0.9985		
Pred. R^2		0.9969			0.7963			0.9396			0.9993		
C.V. %		0.0255			0.2722			0.4409			0.0266		

Emulsion Stability

The RSM as revealed in Table 6 and graphically in Fig. 6 demonstrated the association amongst ES and three factors i.e. NFDM, MCC and OO. The relationship as per equation as follows:

Emulsion Stability = 87.49+0.513A+0.3263B-0.0325C-0.1075AB+0.1350AC- 0.3200BC-0.4198A^2-0.2248B^2-0.4572C^2

ANOVA of the regression coefficient model showed that the model was significant (p<0.05) with p values of <0.0001. The R^2 value (0.9985) expressed that 99.85 % of the total variation reveals a more significant (p<0.05) effect of the factors on the emulsion stability of the low-fat pork patties. In this NFDM, MCC and OO had a significant quadratic and interactive impact on the emulsion stability. The NFDM and MCC had a significant linear effect while OO had non-significant linear impact on the ES.

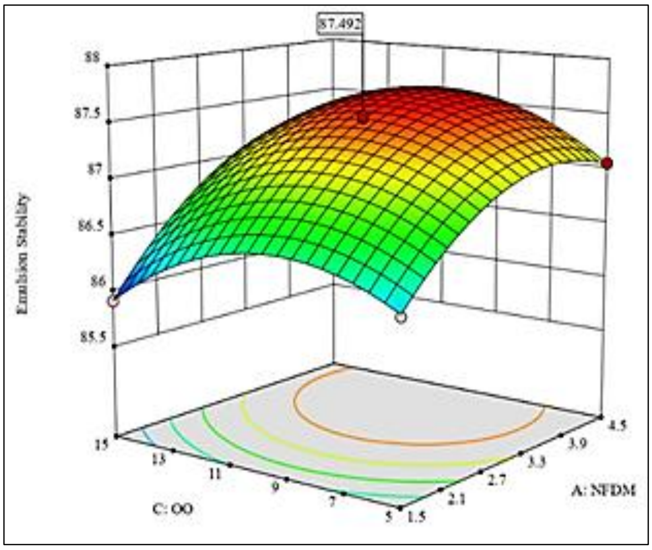

Fig 6: 3D Response surface plot for emulsion stability

Fig. 6 showed the combined effect of NFDM, MCC and olive oil on the emulsion stability of developed low-fat pork patties. It was found that the supply of NFDM increased the emulsion stability of developed low-fat pork patties due to the high protein amount related to NDFM (Kurt & Zorba, 2005) [20]. Proteins adsorbed at interfaces in oil in water emulsion such that hydrophilic amino acid residues were oriented toward the aqueous phase, whereas the hydrophobic segments of the protein molecules were attached to the apolar or lipid phase and the net result had a reduction in the free energy of the system that contributes to emulsion stability (Modler, 1985) [23]. Kurt & Zorba (2005) [20] found that the addition of the NFDM to the meat of different species resulted in increased emulsion stability.

The 3D graph displayed that by raising MCC levels from 1% to 3% ES of the developed low-fat pork patties increased significantly (p<0.05). The reason due to the presence of the hydroxyl-groups (free) which were hydrophilic, while the hydrophobic part on crystalline region resulted in its amphiphilic-nature (Nsor-Atindana *et al*., 2017) [28], due to which it adsorbs well at the oil-water interface and prevents the coalescence of the oil droplets and stabilize the emulsion (Costa *et al*., 2019) [7].

It was discovered that increasing the amount of Olive Oil in the emulsion improved its stability. The explanation for this could be that replacing animal fat (which has a high melting point) with vegetable oil (which has a low melting point) allows for more equal dispersion of oil droplets in the meat emulsion, resulting in a more stable and homogenous protein-water-fat matrix (Kumar *et al*., 2017) [19]. Nieto *et al* (2017) [27] observed similar results in a trial where Olive Oil applied to low-fat frankfurters boosted emulsion stability.

Juiciness

RSM as shown in Table 5 and graphically in Fig. 7 showed the interaction between NFDM, MCC and OO as per the equation as follows:

Juiciness = 7.53-0.0112A + 0.0325B - 0.2263C + 0.0400AB -0.1375AC - 0.0250BC -0.2587A^2 - 0.0762B^2 - 0.0438C^2

The model was significant (p<0.05) with p value 0.0001 for juiciness as per the ANOVA of the regression coefficient.

The lack of fit value 0.1499 revealed the non- significant relation to pure error. The R^2 value (0.9360) expressed that 93.60 % of the total variation reveals more significant ($p<0.05$) effect of the variable on the juiciness of the low-fat pork patties. The statistical analysis revealed that olive oil had significant linear effect on the juiciness of the patties. The NFDM and MCC showed non-significant linear effect on the juiciness of patties. The quadratic effect of NFDM and olive oil showed significant effect on the juiciness value. The interactive effect of NFDM and MCC had significant effect while interactive effect of olive oil showed non-significant effect on the juiciness of developed low- fat pork patties.

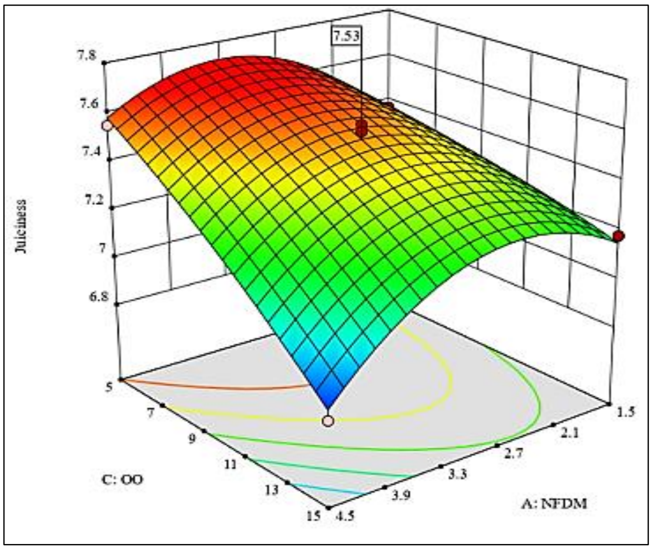

Fig 7: 3D Response surface plot for Juiciness

The fig 7demonstrated the effect of NFDM, MCC and olive oil on the juiciness of developed low-fat pork patties. Linear increase in MCC levels significantly ($p<0.05$) increased the juiciness of developed low-fat pork patties. Similar results were reported by Gibis *et al* (2015) [11] on fried beef patties, MCC addition showed better sensory attributes regarding texture and juiciness than the controls and described that perception of mouthfeel as that of fat-like was enhanced by incorporation of MCC. 3D graph showed that incorporation of NFDM increased the juiciness of developed low-fat pork patties. Kumar & Sharma (2003) [18] postulated that addition of skim milk powder resulted in increased juiciness in the restricted buffalo meat blocks. Increasing levels of olive oil from 3 to 11% resulted in decreased juiciness score in the developed low-fat patties. Delgado-Pando *et al* (2010) [9] reported that addition of olive oil in low fat frankfurters resulted decreased juiciness scores.

The optimum juiciness scores for the low-fat pork patties were recorded as 7.53 as predicted by RSM by the use of three independent variables: NFDM (3%), MCC (2%) and olive oil (7%).

Overall Acceptability

The results pertaining to the overall acceptability of the low-fat pork patties affected by different concentration of MCC, NFDM and olive oil are presented in ANOVA (Table 5) and graphically in Fig. 8 The second order polynomial equation generated relating to overall acceptability and independent variables as follows;

$$OA= 8.06 +0.3062A- 0.1212B- 0.3375C + 0.0325AB+0.5700AC-0.2300BC-0.0618A^2 -0.2818B^2-0.5092C^2$$

The model was significant with value 0.0053 for OA as per the ANOVA of the regression coefficient. The lack of fit value 0.0590 revealed the non-significant relation to pure error. The R^2 value (0.8053) expressed that 80.53 % of the total variation reveals a more significant ($p<0.05$) effect of the variable on the overall acceptability of the low-fat pork patties. The statistical analysis revealed that NFDM and olive oil had a significant linear effect on the overall acceptability of the patties. The linear effect of MCC on overall acceptability was not significant ($p>0.05$). It was evident from ANOVA table that NFDM and MCC had non-significant quadratic effect on OA.

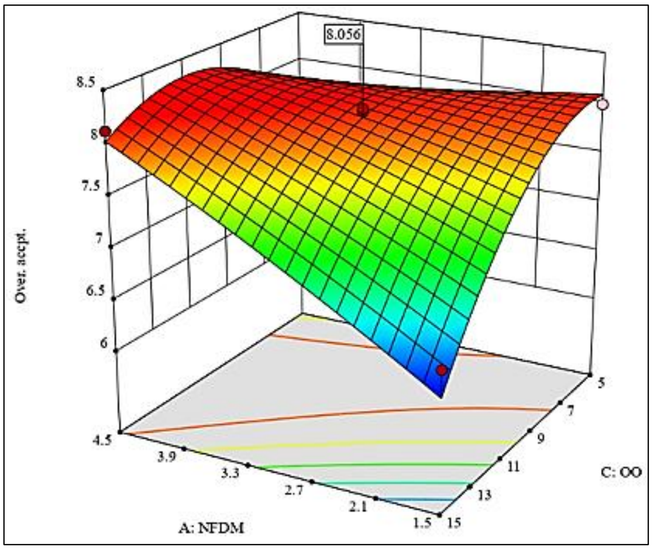

Fig 8: 3D Response surface plot for Overall acceptability

3D graph demonstrated the effect of MCC, NFDM and Olive Oil on the overall acceptability of developed low-fat pork patties. It was observed that the addition of MCC and NFDM increased the overall acceptability of the developed pork patties significantly ($p<0.05$), which are in consonance to study reported by Barbut & Mittal (1996) [4] in development of low-fat frankfurters using microcrystalline cellulose as fat replacer. Fig 8 pointed out that increasing the level of olive oil decreased the overall acceptability in developed low-fat pork patties, which might be due to decrease in characteristic meaty flavour imparted by fork fat as fat is being replace by olive oil. Similar findings were reported by Pappa *et al* (2000) [34] in which complete pork backfat replaced by olive oil resulted in decreased overall acceptability of developed low-fat frankfurters. However, optimum overall acceptability value for the low-fat pork patties was recorded as 7.62 as predicted by RSM by the use of three independent variables: NFDM (3%), MCC (2%) and olive oil (7%)

Conclusion

Low-fat pork patties can be designed by incorporating microcrystalline cellulose, non-fat dry milk powder and olive oil with successful implementation of response surface methodology and using Box-Behnken Design. Standardized protocol of response surface methodology suggested that low-fat pork patties can be developed by the inclusion of MCC at 2 %, NFDM 3% and Olive oil 7% with greater

Cooking Yield, Emulsion Stability and Overall Acceptability.

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